CYTOLOGICAL STUDIES ON NORTH AMERICAN SPECIES OF *SACCHARUM* (POACEAE: ANDROPOGONEAE)

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ABSTRACT

Cytology of the *Saccharum* L. (including *Erianthus* Michx.) species native to North America has not been well characterized. Our objectives were to determine chromosome numbers of 60 clones representative of the eight native species and varieties and attempt crosses with elite sugarcane (*Saccharum* spp. hybrids). Counts are reported for the first time for *S. brevibarbe* var. *brevibarbe* (2n = 60), *S. coarctatum* (2n = 60), and *S. giganteum* (2n = 30, 60, and 90). The latter represents the first report of a polyploid series within *S. giganteum* and the first count of 2n = 90 for the *Erianthus* group. Counts also were made for *S. alopecuroideum* (2n = 30), *S. baldwinii* (2n = 30), and *S. brevibarbe* var. *contortum* (2n = 60). Five putative crosses were made between sugarcane hybrids and native *Saccharum*, yielding 4 to 448 seeds per cross.

RESUMEN

La citología de las especies de *Saccharum* L. (incluyendo *Erianthus* Michx.) nativas de Norte América no ha sido bien caracterizada. Nuestros objetivos fueron determinar el número cromosómico de 60 clones representativos de ocho especies y variedades nativas e intentar cruces con caña de azúcar de élite (híbridos de *Saccharum* spp.). Se ofrecen por primera vez recuentos de *S. brevibarbe* var. *brevibarbe* (2n = 60), y *S. coarctatum* (2n = 60), y *S. giganteum* (2n = 30, 60, y 90). Este último representa la primera cita de una serie poliploide en *S. giganteum* y el primer recuento de 2n = 90 para el grupo *Erianthus*. Se hicieron también recuentos de *S. alopecuroideum* (2n = 30), *S. baldwinii* (2n = 30), y *S. brevibarbe* var. *contortum* (2n = 60). Se hicieron cinco cruces putativos entre híbridos de caña de azúcar y *Saccharum* silvestres, produciendo de 4 a 448 semillas por cruce.

INTRODUCTION

There has been disagreement among taxonomists concerning the treatment and placement of *Saccharum* L. and *Erianthus* Michx. A brief history of the vari-

SIDA 16(2): 233 – 244. 1994

ous concepts of these genera is presented in Webster & Shaw (1995). North American taxonomists have followed the concepts of Hitchcock (1951) and have recognized both genera. European agrostologists have placed *Erianthus* within *Saccharum*. Characteristics used to distinguish the genera are difficult to apply when the full range of variation is considered. Therefore, we are following the concept presented in Clayton & Renvoize (1986) that recognizes *Erianthus* as a synonym of *Saccharum*.

Sugarcane breeders recognize separate genera and generally include five to six species within *Saccharum*: *S. edule* Hassk. (2n = 60 to 80), *S. officinarum* L. (2n = 80), *S. robustum* Brandes & Jesw. ex Grassl (2n = 60, 80, to about 200), *S. sinense* Roxb. (2n = 111 to 120), and *S. spontaneum* L. (2n = 40 to 128). A sixth species, *S. barberi* Jesw. (2n = 81 to 124), is sometimes included in *S. sinense*. Chromosome counts (Daniels et al. 1975) for closely related taxa called the "*Saccharum* complex" by Mukerjee (1957) include Old World *Erianthus* Michx. sect. *Ripidium* Henrard (2n = 20, 30, 40, 60); *Miscanthus* Anderss. sect. *Diandra* Keng (2n = 40); *Narenga* Bor (2n = 30); and *Sclerostachya* (Hack.) A. Camus (2n = 30). These and a few other genera have at times been placed in *Saccharum* (Daniels and Roach 1987; Whalen 1991). The basic genomes within *Saccharum* (s. str.) appear to be x = 8, 10, and 12 (Sreenivasan et al. 1987), and that of *Erianthus* may be x = 5, typical of the Andropogoneae (Celarier 1956a).

Harlan and de Wet (1975) noted that *Saccharum* had an "oversplit taxonomy," implying that many taxonomic divisions may be artificial. As evidence of this, interspecific and intergeneric crosses within the *Saccharum* complex are usually successful (Gill and Grassl 1986; Grassl 1980; Daniels and Roach 1987). The taxonomic relationships among the taxa of the *Saccharum* complex have been neither carefully studied nor well-defined (Webster and Shaw 1995) and they conclude that *Erianthus* is best treated as a synonym of *Saccharum*. Five species and one variety of *Saccharum* L. were recognized by Webster and Shaw (1995) as being native to North America. They are *S. alopecuroideum* (L.) Nutt. [= *Erianthus alopecuroides* (L.) Ell.], *S. baldwinii* Spreng. (= *E. strictus* Baldw.), *S. brevibarbe* (Michx.) Pers. var. *brevibarbe*, *S. brevibarbe* (Michx.) Pers. var. *contortum* (Nutt.) R. Webster (= *E. contortus* Ell.), *S. coarctatum* Fern. (= *E. coarctatus* Fern.), and *S. giganteum* (Walt.) Pers. [= *E. giganteus* (Walt.) C.E. Hubb.].

Old World species previously treated in *Erianthus* (sect. Ripidium) are used in sugarcane breeding (Heinz 1991), particularly for its disease resistance (Burner et al. 1993; Grisham et al. 1992) and freeze tolerance (Moore 1987). Chromosome numbers of many clones have been reported (Babu and Srinivasan 1960; Mohan and Sreenivasan 1983). Hybrids between *Saccharum* spp. and North American species placed in *Erianthus* have not been reported, although Gill and Grassl (1986) reported hybrids between *Sclerostachya fusca* (Roxb.) A. Camus and *E. brevibarbis* Michx. (= S. *brevibarbe*), *E. tracyi* Nash. (= *S. alopecuroideum*), *E.*

contortus Baldw., and between *Miscanthus* sp. and *E. brevibarbis* Michx. (= *S. brevibarbe*).

Except for the few clones (five clones in four species) reported by Celarier (1956b) and Gill and Grassl (1986), there have been no cytological studies of the North American species traditionally placed in *Erianthus*. The objectives of this research were to determine chromosome numbers and characteristics in a complete collection of North American species of these taxa and attempt hybridization with elite sugarcane hybrids (interspecific and intergeneric *Saccharum* spp. hybrids).

MATERIALS AND METHODS

Clones were collected as rhizomes from natural populations in the mid-Atlantic and southeastern U.S.A. (Fall 1992) and southcentral U.S.A. (Fall 1993) and were grown at Houma, Louisiana (29° 35'N 90° 44'W) in 18.9 L cans filled with a soil mix of 3:2:2 (soil:sand:peat moss). Taxonomy was verified according to the concepts presented in Webster and Shaw (1995). Voucher specimens are deposited at TAES.

Inflorescences were collected at early boot stage and fixed in Carnoy's B: ethanol, acetic acid, and chloroform (6:3:1 mixture by volume) (Smith 1947) and a few drops of saturated ferric chloride. Anthers were squashed in 5 g L^{-1} propionocarmine. Chromosome number was determined from pairing configurations at diakinesis or metaphase I (MI) in 5 to 25 microsporocytes per plant. Chromosome number for some plants was determined or verified in squashes of root tip cells using standard procedures. Pollen stainability, an estimate of pollen viability, was measured by staining mature anthers in 10 g L^{-1} I_2-KI.

The cross-sectional area of individual bivalent chromosomes was measured at MI for each of nine clones (range 1 to 15 cells $clone^{-1}$, mean 6 cells $clone^{-1}$) using a Cue-2[1] image analyzer (Galai 1990). Area (μm^2) was determined from the number of pixels in the bivalent chromosome.

About 100 completely filled pollen grains from mature anthers of 25 plants were collected in Fall 1993, stained in 10 g L^{-1} I_2-KI, and imaged at 400x. Cross-sectional area was calculated as described above. Average cross-sectional radius (μm) of each pollen grain was the mean of eight Martin's radii measured at 0, 45, 90, 135, 180, 225, 270, and 315° (Galai 1990). Volume (μm^3) was calcu-lated from average radius assuming that pollen grains were perfect spheres.

Analysis of variance of pollen area and volume was done by the general linear models procedure (SAS Institute 1990). Sources of variation were species (7 df),

[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

clone within species (16 df), and residual (2,773 df). The effect of species was tested using clone within species as error; clone within species was tested using residual as error. Means were compared using least significant difference at the 5% level of significance (Steel and Torrie 1980). The cluster procedure (SAS Institute 1990) was used to assign clones to non-overlapping hierarchical groups based on multivariate analysis of chromosome number, pollen area, and pollen volume. Prior to conducting the cluster analysis, data were standardized to a mean of 0 and standard deviation of 1 using procedure "standard" (SAS Institute 1990).

Crosses between the native North American taxa of *Saccharum* and elite sugarcane clones, cytoplasmically male-sterile (cms) *Sorghum bicolor* (L.) Moench, and *Vetiveria zizanioides* (L.) Nash (used as females) were attempted in Fall 1993. Flowering of sugarcane clones was induced using standard photoperiod treatment (Dunckelman and Legendre 1982). Flowering of *Sorghum*, North American *Saccharum*, and *V. zizanioides* occurred under natural photoperiod. Conventional methods of crossing, seed maturation, seed germination, and seedling establishment were used (Dunckelman and Legendre 1982).

RESULTS AND DISCUSSION

Chromosome number varied among and within the North America *Saccharum* species (Table 1). *Saccharum alopecuroideum* and *S. baldwinii* were 2n = 30. Counts of 2n = 30 in *E. strictus* from Texas (Celarier 1956b) and *E. tracyi* (Gill and Grassl 1986) are consistent with our data. A count of 2n = 60 in *S. alopecuroideum* (Celarier 1956b) is inconsistent with our data. However, two clones of *S. giganteum* (2602 and 2603) with 2n = 60 and characteristics similar to *S. alopecuroideum* were collected in Tennessee and Arkansas. It seems probable that Celarier's count of 2n = 60 may be based on *S. giganteum* according to present taxonomic concepts. Clones with 2n = 30 and 2n = 60 are probably 6*x* and 12*x*, respectively.

Saccharum giganteum consisted of clones with 2n = 30, 60, and 90 chromosomes. These are the first counts for the species, the first indication that the species is a polyploid series, and the first report of 2n = 90 (18x) in the taxa traditionally placed in *Erianthus*. The 2n = 30 types were collected from Maryland south to Alabama and in southern Louisiana; the 2n = 60 types were collected in Alabama, Arkansas, Georgia, Louisiana, and Tennessee; and the 2n = 90 types were collected in southern Georgia and Florida. This indicates a geographic effect on distribution of cytotypes. Future studies are planned to define the relationship between chromosome number and morphology within this species. *Saccharum bengalense* Retz. [= *E. bengalense* (Retz.) Bharadw.] has also been shown to be a polyploid series with 2n = 20, 40, and 60 chromosomes (Mehra et al. 1968).

TABLE 1. Chromosome numbers and collection sites of native North American species of *Saccharum*.

S. alopecuroideum

2n = 30: ALABAMA. **Blount Co.:** Hwy 278, 32 km W of Gadsden, 6 Nov 1992, *Webster 2551* (TAES). **Lamar Co.:** Hwy. 18, 24 km E of Fayettsville, 6 Nov 1992, *Webster 2548* (TAES). GEORGIA. **Banks Co.:** Interstate 85, 3 km E of Commerce, 7 Nov 1992, *Webster 2553* (TAES). **Brooks Co.:** Hwy 84, 8 km E of county road 219, 27 Oct 1992, *Webster 2533* (TAES). **Forsyth Co.:** Hwy 9, 6 km S of Cummings, 7 Nov 1992, *Webster 2552* (TAES). MISSISSIPPI. **Choctaw Co.:** Natchez Trace Pkwy., 13 km S of Hwy 9, 28 Oct 1992 *Webster 2544* (TAES). TENNESSEE. **Putnam Co.:** Interstate 40, just E of mile marker 293, 8 Nov 1993, *Webster 2600* (TAES).

S. baldwinii

2n = 30: ALABAMA. **Tuscaloosa Co.:** Hwy 82, 8 km N of Tuscaloosa, 28 Oct 1992, *Webster 2541* (TAES). GEORGIA. **Charlton Co.:** Hwy 23 & 121, 8 km S of Folson, 26 Oct 1992, *Webster 2525* (TAES). **Lowndes Co.:** Hwy 84, 8 km W of Valdosta, 27 Oct 1992, *Webster 2532* (TAES).

S. brevibarbe var. *brevibarbe*

2n = 60: ARKANSAS. **Perry Co.:** Hwy 10, 2 km E of Hwy 324 jct, 10 Nov 1993, *Webster 2607* (TAES). **White Co.:** Hwy 367, within Garner city limits, 9 Nov 1993, *Webster 2605* (TAES).

S. brevibarbe var. *contortum*

2n = 60: ALABAMA. **Autauga Co.:** Hwy 82, 2 km E of county road 29, 28 Oct 1992, *Webster 2538* (TAES). **Chilton Co.:** Hwy 82, 7 km S of county road 65, 28 Oct 1992, *Webster 2539* (TAES). **Houston Co.:** Hwy 84, at AL state line, 27 Oct 1992, *Webster 2536* (TAES). **Lamar Co.:** Hwy 96, 8 km from MS state line, 6 Nov 1992, *Webster 2547* (TAES). **Pickens Co.:** Hwy 82, 13 km from MS state line, 28 Oct 1992, *Webster 2542* (TAES). ARKANSAS. **White Co.:** Hwy 64, 6 km W of Beebe, 10 Nov 1993, *Webster 2606* (TAES). **Yell Co.:** Hwy 10, 2 km W of Birta, 10 Nov 1993, *Webster 2608* (TAES). GEORGIA. **Decatur Co.:** Hwy 84, 1 km from jct 285, 14 km E of Donaldsonville, 27 Oct 1992, *Webster 2535* (TAES). MARYLAND. **Somerset Co.:** Hwy 13, S of Salisbury, 23 Oct 1992, *Webster 2502* (TAES); Hwy 13, 11 km N of Pocomoke, 23 Oct 1992, *Webster 2503* (TAES). MISSISSIPPI. **Choctaw Co.:** Natchez Trace Pkwy., 2 km S of Hwy 9, 28 Oct 1992, *Webster 2543* (TAES). NORTH CAROLINA. **Greene Co.:** Hwy 13, 16 km S of Greenville, 24 Oct 1992, *Webster 2509* (TAES). **Halifax Co.:** Hwy 258, 2 km N of Hwy 97, 24 Oct 1992, *Webster 2508* (TAES). SOUTH CAROLINA. **Florence Co.:** Hwy 301, 3 km N of Langston Rd, 25 Oct 1992, *Webster 2514* (TAES). TEXAS. **Angelina Co.:** Hwy 69, 6 km N of 7 jct., 13 Nov 93, *Webster 2611* (TAES). **Cherokee Co.:** Hwy 175, 6 km W of Jacksonville, 13 Nov 93, *Webster 2609* (TAES). VIRGINIA. **Accomack Co.:** Hwy 13, 91 m off of Parkway, 2 km N of Keller, 23 Oct 1992, *Webster 2504* (TAES).

S. coarctatum

2n = 60: ALABAMA. **Henry Co.:** Hwy 95, 29 km N of state line, 27 Oct 1992, *Webster 2537* (TAES). FLORIDA. **Clay Co.:** Hwy 301, 27 km N of Starke, 26 Oct 1992, *Webster 2527* (TAES). GEORGIA. **Brantley Co.:** Hwy 301, 14 km N of Nahunta, 26 Oct 1992, *Webster 2523* (TAES). **Evans Co.:** Hwy 301, 8 km N of Canoochee River, 25 Oct 1992, *Webster 2520* (TAES). **Grady Co.:** Hwy 84 & Steward Road, 27 Oct 1992, *Webster 2534* (TAES). **Screven Co.:** Hwy 301, 8 km S of state line, 25 Oct 1992, *Webster 2519* (TAES). **Wayne Co.:** Hwy 301, 3 km N of Jones Creek, Bethel Church, 25 Oct 1992, *Webster 2522* (TAES). SOUTH CAROLINA. **Dillon Co.:** Hwy 301, Little Pee Dee River, 8 km S of the state line, 24 Oct 1992, *Webster 2513* (TAES). **Clarendon Co.:** Hwy 301, 1 km N of Fox Tindal Road, 25 Oct 1992, *Webster 2516* (TAES). **Orangeburg Co.:** Hwy 301, 11 km E of Orangeburg, 25 Oct 1992, *Webster 2517* (TAES).

S. giganteum

2n = 30: ALABAMA. **Bibb Co.:** Hwy 82, 2 km from jct Hwy 91, 28 Oct 1992, *Webster 2540* (TAES). FLORIDA. **Gilchrist Co.:** Hwy 26, 13 km E of Trenton, 26 Oct 1992, *Webster 2528* (TAES).

Table 1. Continued

LOUISIANA. **Terrebonne Parish:** Spanish Trail Farm, Chacahoula, *Burner 2554* (TAES). NORTH CAROLINA. Robeson Co.: Hwy 301, 5 km N of Little Marsh Swamp, 24 Oct 1992, *Webster 2512* (TAES). **Wayne Co.:** Hwy 13, 2 km N of Rachel Jackson Hwy, 24 Oct 1992, *Webster 2510* (TAES). MARYLAND. **Wicomico Co.:** Hwy 50, just W of exit 331, 23 Oct 1992, *Webster 2501* (TAES). SOUTH CAROLINA. **Bamberg Co.:** Hwy 301, 11 km S of Bamberg, 25 Oct 1992, *Webster 2518* (TAES). **Clarendon Co.:** Hwy 301, 2 km N of Manning, 25 Oct 1992, *Webster 2515* (TAES). VIRGINIA. **Accomack Co.:** Hwy 13, 3 km N of Hwy 704, 23 Oct 1992, *Webster 2505* (TAES). **Southampton Co.:** Hwy 258, 3 km N of NC state line, 24 Oct 1992, *Webster 2506* (TAES).

2n = 60: ALABAMA. **Cullman Co.:** Hwy 91, at 4 mile marker, 6 Nov 1992, *Webster 2550* (TAES). **Lamar Co.:** Hwy 50, at AL state line, 6 Nov 1992, *Webster 2546* (TAES). **Walker Co.:** Hwy 69, 3 km S of Jasper, 6 Nov 1992, *Webster 2549* (TAES). ARKANSAS. **White Co.:** Hwy 367, 91 m E of Bradford city limits, 9 Nov 1993, *Webster 2603* (TAES). GEORGIA. **Evans Co.:** Hwy 301, 8 km N of Canoochee River, 25 Oct 1992, *Webster 2521* (TAES). LOUISIANA. **St. Landry Parish:** Hwy 49, 53 km N of Opelousas, 30 Oct 1992, *Webster 2545* (TAES). TENNESSEE. **Carroll Co.:** Hwy 70A bypass, N of Huntington, 8 Nov 1993, *Webster 2602* (TAES).

2n = 90: FLORIDA. **Dixie Co.:** Hwy 19 & 98, 2 km S of county road S358, 26 Oct 1992, *Webster 2529* (TAES). **Taylor Co.:** Hwy 51, 10 km from the Gulf of Mexico, 26 Oct 1992, *Webster 2530* (TAES); Hwy 221, 6 km N of Perry, 27 Oct 1992, *Webster 2531* (TAES). GEORGIA. **Charlton Co.:** Hwy 23 & 121, 2 km N of the FL line, 26 Oct 1992, *Webster 2526* (TAES).

Saccharum brevibarbe vars. *brevibarbe* and *contortum* and *S. coarctatum* were 2n = 60. Gill and Grassl (1986) reported that a clone of *E. brevibarbis* was 2n = 60, which confirmed our finding for the species. However, they reported that a clone of *E. contortus* was 2n = 30, which may be the result of misidentification. *Saccharum brevibarbe* var. *contortum* is characterized as having callus hairs equal to or shorter than the spikelet, while callus hairs are either absent (*S. baldwinii*) or longer (*S. giganteum* and *S. alopecuroideum*) than the spikelet in 2n = 30 types (Webster and Shaw 1995).

Meiosis was normal in all clones and univalents and quadrivalents were extremely rare (Fig. 1). Celarier (1956b) noted bivalent size polymorphism in North American *Saccharum*. Mean bivalent area of 4.43 μm^2 (range 1.58 to 8.94 μm^2), 3.25 μm^2 (range 1.10 to 8.77 μm^2), and 4.43 μm^2 (range 1.86 to 7.61μm^2) in 2n = 30, 60, and 90 biotypes, respectively, differed little among cytotypes. Bivalent area was not normally distributed; the distribution was generally shifted toward the smaller size classes. Bivalents of an Old World clone (*E. rufipilus*, 2n = 20) averaged 3.89 μm^2 (range 3.25 to 5.28 μm^2), and those of an elite sugarcane clone LCP 81-30 (2n = 105, 108) averaged 3.42 μm^2 (range 1.55 to 6.99 μm^2) (Burner and Legendre 1994; Burner unpublished data). Thus, North American *Saccharum* tends to have wider ranges of bivalent area than Old World *Saccharum*, but there is little difference in area or range between New World *Saccharum* and sugarcane. The data support the observation by Gould (1956) that there is no obvious correlation between chromosome number and chromosome size in the Andropogoneae.

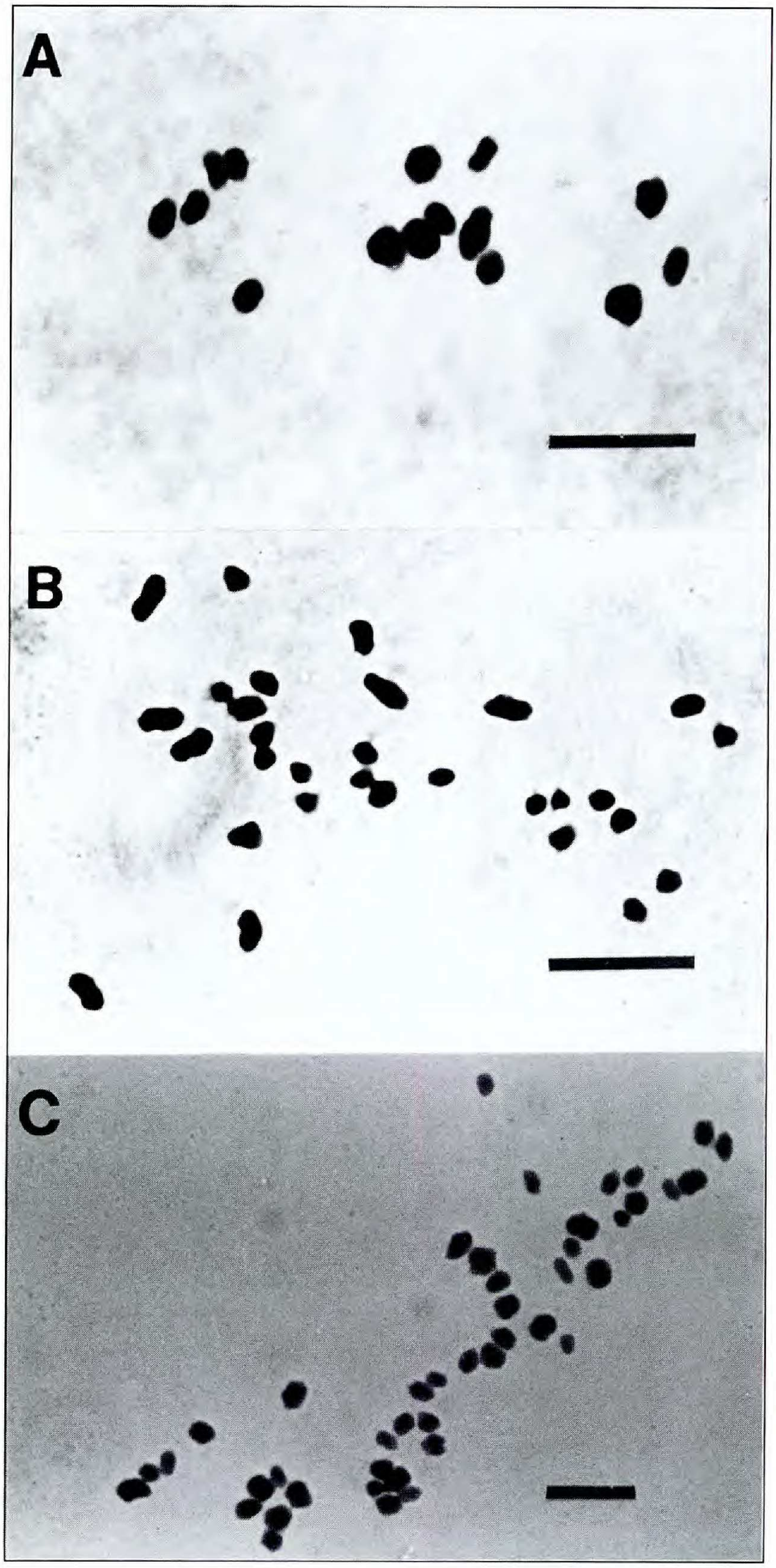

FIG. 1. Meiotic metaphase I chromosomes of North American *Saccharum*. Bar in each figure represents 10 μm. (A) *S. giganteum* (n = 15 bivalents) [×1600]. (B) *S. brevibarbe* var. *contortum* (n = 30 bivalents) [×1600]. (C) *S. giganteum* (n = 45 bivalents) [×1000].

Pollen area and volume differed significantly among species and clones within species (Table 2). As expected, species with 2n = 30 had smaller pollen than those with 2n = 60 or 2n = 90 (Gould 1957). Pollen size has been used as a taxo-nomic character to separate closely related taxa differing in chromosome number (Gould 1953 & 1957). However, variation in pollen size among clones within species indicates that pollen size would be an unreliable predictor of species.

Clones were assigned to clusters based on chromosome number, pollen area, and pollen volume. The dendrogram beginning with seven clusters ($R^2 = 0.97$) and ending with one cluster ($R^2 = 0.00$) is shown in Fig. 2. There was a

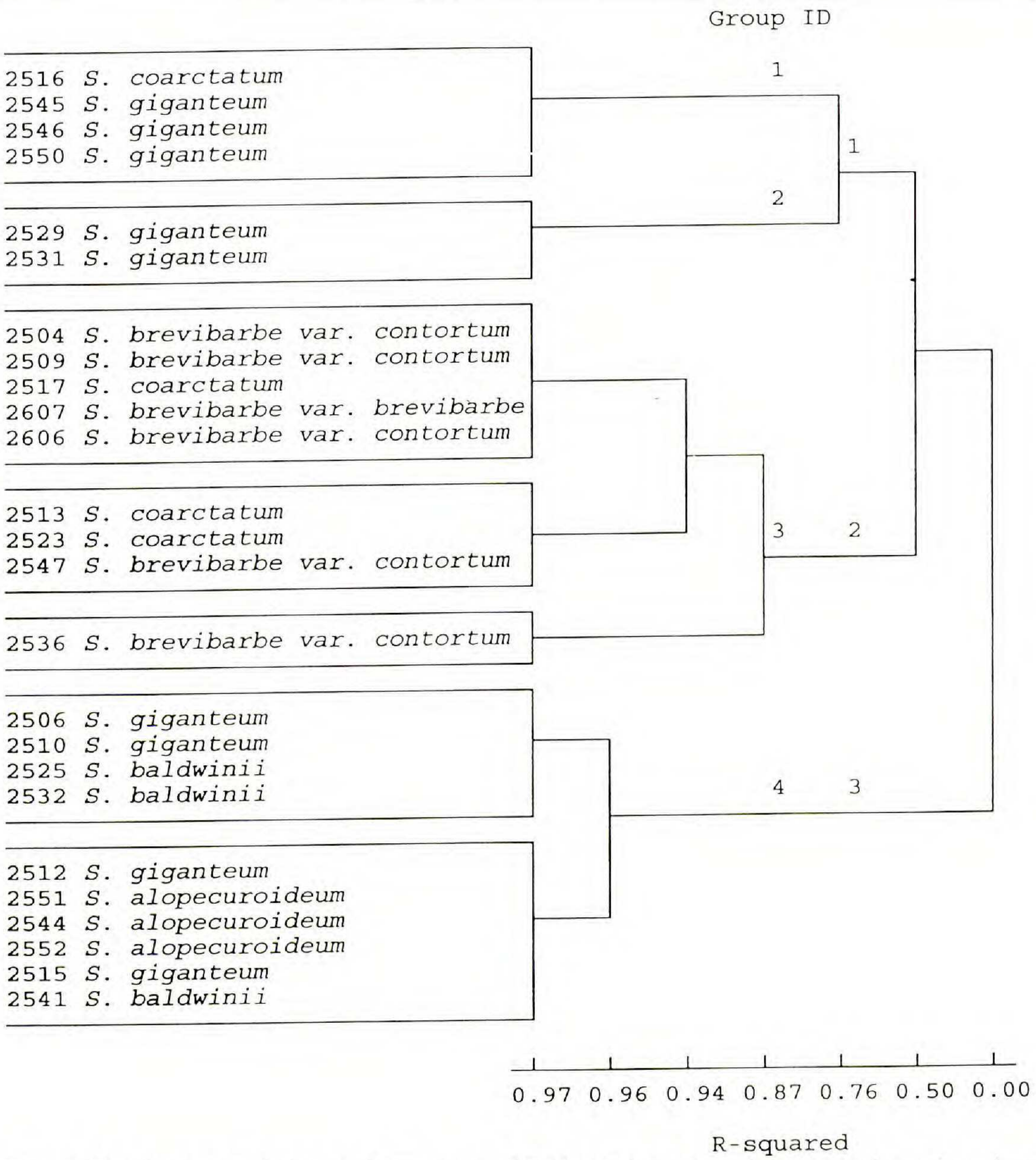

FIG. 2. Dendrogram of relationships among 25 North American *Saccharum* clones based on pollen area, pollen volume, and chromosome number.

TABLE 2. Mean pollen area and pollen volume of North American *Saccharum* clones.

Species/clone	Pollen	
	Area (μm^2)	Volume (μm^3)
S. alopecuroideum	1052	25761
2544	1028	24840
2551	1044	25421
2552	1084	27022
S. baldwinii	1218	32146
2525	1282	34478
2532	1243	33248
2541	1129	28712
S. brevibarbe var. ***brevibarbe***	1926	63635
2607	1926	63635
S. brevibarbe var. ***contortum***	2020	68814
2504	1854	59960
2509	1914	63254
2536	2476	92818
2547	1752	55298
2606	2106	72741
S. coarctatum	1751	55459
2513	1711	53577
2516	1603	48565
2517	1924	63544
2523	1765	56152
S. giganteum 2n = 30	1184	30987
2506	1271	34365
2510	1277	34685
2512	1045	25494
2515	1144	29405
S. giganteum 2n = 60	1447	41065
2545	1529	45172
2546	1417	40494
2550	1394	39148
S. giganteum 2n = 90	1478	43090
2529	1447	41883
2531	1510	44297
Mean	1511	45366
CV (%)	12.3	18.9
LSD (0.05) - Species	207	10324
LSD (0.05) - Clone within species	52	2397

TABLE 3. Seed yield of crosses with North American *Saccharum*.

Cross no.	Parents		Viable seeds (no.)
	Female	Male	
3003	A4692[1]	2508	0
3004	A_3TX7000[1]	2535	0
3006	A_2TX632[1]	2504	2
3007	A83E[1]	2551	1
3008	ATX 623[1]	2525	0
3011	POJ 2222[3]	2543	26
3031	IND 81-53[2]	2532	0
3144	CP 65-357[3]	2531	448
3145	CP 91-534[3]	2531	92
3206	CP 92-670[3]	2530	12
3209	CP 65-357[3]	2533	4
3210	CP 88-755[3]	2533	0

[1] CMS *Sorghum*.
[2] *Vetiveria zizanioides* 2n = 20 (D.M. Burner, unpublished data).
[3] Elite sugarcane clone.

10% loss in explained variance when seven clusters were combined to four clusters. Four-cluster analysis (R^2 = 0.87) seemed to be most informative. Clones of *S. giganteum* (2n = 60) and *Webster 2516* (*S. coarctatum*, 2n = 60) were assigned to cluster group 1. Morphology of *Webster 2516* is otherwise typical of *S. coarctatum*. Clones of *S. giganteum* with 2n = 90 chromosomes were assigned to cluster group 2. Clones of *S. brevibarbe* vars. *brevibarbe* and *contortum* and *S. coarctatum*, except for *Webster 2516* were assigned to cluster group 3. Clones with 2n = 30 were assigned to cluster group 4. Thus, the four-cluster analysis was generally consistent with present taxonomic concepts (Webster and Shaw 1995) and provided evidence of diversity between the cytotypes of *S. giganteum*. There was a further loss of 11% of explained variance when the 2n = 60 and 2n = 90 cytotypes of *S. giganteum* were joined to form three clusters. The three-cluster analysis explained 76% of variance. Only 50% of total variance was explained by two clusters.

Crosses were successful between elite sugarcane and North American *Saccharum* (Table 3). A cross with *Webster 2531* (2n = 90) yielded 448 seeds, and other crosses yielded 0 to 92 seeds. The potential agronomic value of these F_1 hybrids will be evaluated in subsequent tests. Two crosses of cms *Sorghum* × North American *Saccharum* (five crosses attempted) yielded some seed, but grow-out evaluation showed that the F_1 progeny were not hybrid. Crosses of cms *Sorghum* × elite sugarcane also failed to yield hybrid progeny (Burner unpublished data). *Vetiveria zizanioides* (2n = 20) × *Webster 2532* (2n = 30) was also unsuccessful. Pistillate sterility is frequently observed in *V. zizanioides* (Ramanujam and Kumar

1963), and despite several attempts we have never obtained viable seeds from this species.

Löve (1951) noted that differing chromosome numbers within a species, as we found in *S. giganteum*, indicates the species may include more than one distinguishable taxon and needs closer taxonomic inspection. Löve (1951) and Nannfeldt (1938) further argue that intraspecific difference in ploidy level has fundamental systematic value sufficient to justify the recognition of their respec-tive members as species. Cytomorphological study of *S. giganteum* should be conducted and crosses between 6*x* and 18*x* forms, to attempt synthesis of the intermediate 12*x* form, would further our understanding of the evolution of the genus.

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REFERENCES

BABU, C.N. and K. SRINIVASAN. 1960. Chromosome numbers in the genus *Erianthus*. Sci. Cult. 26:230–231.

BURNER, D.M., M.P. GRISHAM, and B.L. LEGENDRE. 1993. Resistance of sugarcane relatives injected with *Ustilago scitaminae*. Plant Dis. 77:1221–1223.

BURNER, D.M. and B.L. LEGENDRE. 1994. Cytogenetic and fertility characteristics of elite sugarcane clones. Sugar Cane 1994 (1):6–10.

CELARIER, R.P. 1956a. Additional evidence for five as a basic number of the Andropogoneae. Rhodora 58:135–143.

_____. 1956b. Cytotaxonomy of the Andropogoneae I. Subtribes Dimeriinae and Saccharinae. Cytologia 21:272–291.

CLAYTON, W.D. and S.A. RENVOIZE. 1986. Genera graminum: Grasses of the World. Kew, London: Royal Botanical Gardens.

DANIELS, J., P. SMITH, N. PATON, and C.A. WILLIAMS. 1975. The origin of the genus *Saccharum*. Sugarcane Breed. Newsl. 36:24–39.

DANIELS, J. and B.T. ROACH. 1987. Taxonomy and evolution. pp. 7–84. In: D.J. Heinz (ed.) Sugarcane improvement through breeding. Elsevier, New York.

DUNCKELMAN, P.H. and B.L. LEGENDRE. 1982. Guide to sugarcane breeding in the temperate zone. USDA-ARS ARM-S-22. Agric. Res. Serv. Southern Region. USDA, New Orleans, Louisiana.

GALAI PRODUCTION LTD. 1990. Cue-2 image analyzer operation manual, version 3.2. Olympus Corp., Lake Success, New York.

GILL, B.S. and C.O. GRASSL. 1986. Pathways of genetic transfer in intergeneric hybrids of sugar cane. Sugar Cane 1986(2):2–7.

GOULD, F.W. 1953. A cytotaxonomic study in the genus *Andropogon*. Amer. J. Bot. 40:297–306.

_____. 1956. Chromosome counts and cytotaxonomic notes on grasses of the tribe Andropogoneae. Am. J. Bot. 43:395–404.

_____. 1957. Pollen size as related to polyploidy and speciation in the *Andropogon saccharoides-A. barbinodis* complex. Brittonia 9:71–75.

GRASSL, C.O. 1980. Breeding Andropogoneae at the generic level for biomass. Sugarcane Breed. Newsl. 43:41–57.

GRISHAM, M.P., D.M. BURNER, and B.L. LEGENDRE. 1992. Resistance to the H strain of sugarcane mosaic virus among wild forms of sugarcane and relatives. Plant Dis. 76:360–362.

HARLAN, J.R. and J.M.J. de WET. 1975. On Ö Winge and a prayer: The origins of polyploidy. Bot. Rev. 41:361–390.

HEINZ, D.J. 1991. Sugarcane cytogenetics. pp. 279–293. In: T. Tsuchiya and P.K. Gupta (eds.) Chromosome engineering in plants: Genetics, Breeding, Evolution, Part B. Elsevier, Amsterdam.

HITCHCOCK, A.S. 1951. Manual of the grasses of the United States, 2nd ed. (revised by A. Chase). U.S. Dept. Agric., Misc. Publ. No. 200, Washington, D.C.

LÖVE, A. 1951. Taxonomical evaluation of polyploids. Caryologia 3:263–284.

MEHRA, P.N., P.K. KHOSLA, B.L. KOHLI, and J.S. KOONAR. 1968. Cytological studies in the North Indian grasses (part 1). Res. Bull. Punjab. Univ. 19:157–230.

MOHAN, N. and T.V. SREENIVASAN. 1983. Distribution and chromosome number in the genus *Erianthus*. Sugarcane Breed. Newsl. 45:50–63.

MOORE, P.H. 1987. Breeding for stress tolerance. pp. 503–542. In: D.J. Heinz (ed.) Sugarcane improvement through breeding. Elsevier, New York.

MUKHERJEE, S.K. 1957. Origin and distribution of *Saccharum*. Bot. Gaz. 119:55–61.

NANNFELDT, J.A. 1938. *Poa maroccana* Nannf. n. sp. and *P. rivulorum* Maire & Trabut, two more tetraploids of sect. *Ochlopoa* A. & Gr., and some additional notes on *Ochlopoa*. Svensk. Bot. Tidskr. 32:295–321.

RAMANUJAM, S. and S. KUMAR. 1963. Irregular meiosis associated with pollen sterility in *Vetiveria zizanioides* (Linn.) Nash. Cytologia 28:242–247.

SAS INSTITUTE. 1990. SAS/STAT user's guide, version 6, fourth ed. SAS Institute, Cary, North Carolina.

SMITH, L. 1947. The acetocarmine smear technic. Stain Technol. 22:17–31.

SREENIVASAN, T.V., B.S. AHLOOWALIA, and D.J. HEINZ. 1987. Cytogenetics. pp. 211–253. In D.J. Heinz (ed.) Sugarcane improvement through breeding. Elsevier, New York.

STEEL, R.G.D. and J.H. TORRIE. 1980. Principles and procedures of statistics. McGraw-Hill, New York.

WEBSTER, R.D. and R.B. SHAW. 1995. Taxonomy of the native North American species of *Saccharum* (Poaceae: Andropogoneae). Ann. Missouri. Bot. Gard. (submitted)

WHALEN, M.D. 1991. Taxonomy of *Saccharum* (Poaceae). Baileya 23:109–125.